



## Contribution of plants to N<sub>2</sub>O emissions in soil-winter wheat ecosystem: pot and field experiments

Jianwen Zou<sup>1,3</sup>, Yao Huang<sup>1,2</sup>, Wenjuan Sun<sup>1</sup>, Xunhua Zheng<sup>2</sup> & Yuesi Wang<sup>2</sup>

<sup>1</sup>College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing, 210095, P.R. China. <sup>2</sup>LAPC, Institute of Atmospheric Physics, Chinese Academy of Sciences, Beijing, 10029, P.R. China.

<sup>3</sup>Corresponding author\*, present address: Department of Ecology and Evolutionary Biology, Rice University, Houston, TX 77005, USA

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### Abstract

Outdoor pot and field experiments were conducted to assess the role of growing plants in agricultural ecosystem N<sub>2</sub>O emissions. N<sub>2</sub>O emissions from plants were quantified as the difference in soil-crop system N<sub>2</sub>O emissions before and immediately after cutting plants during the main growth stages in 2001–02 and 2002–03 winter wheat seasons. Emissions of N<sub>2</sub>O from plants depended on biomass within the same plant developmental status. Field results indicated that the seasonal contribution of N<sub>2</sub>O emissions from plants to ecosystem fluxes averaged 25%, ranging from 10% at wheat tillering to 62% at the heading stage. The fluxes of N<sub>2</sub>O emissions from plants varied between 0.3 and 3.9 mg N<sub>2</sub>O-N m<sup>-2</sup> day<sup>-1</sup> and its seasonal amount was equivalent to 0.23% of plant N released as N<sub>2</sub>O. A N<sub>2</sub>O emission coefficient (N<sub>2</sub>O<sub>E</sub>, mg N<sub>2</sub>O-N g<sup>-1</sup> C day<sup>-1</sup>), defined as N<sub>2</sub>O-N emission in milligrams from per gram carbon of plant dry matter within a day, was represented by a 5-fold variation ranging from 0.021 to 0.004 mg N<sub>2</sub>O-N g C<sup>-1</sup> day<sup>-1</sup>. A linear relationship ( $y = 0.4611x + 0.0015$ ,  $r^2 = 0.9352$ ,  $p < 0.001$ ) between N<sub>2</sub>O<sub>E</sub> ( $y$ ) and plant dark respiration rate ( $x$ , mg CO<sub>2</sub>-C g C<sup>-1</sup> day<sup>-1</sup>) suggested that in the absence of photosynthesis, some N<sub>2</sub>O production in plant N assimilation was associated with plant respiration. Although this study could not show whether N<sub>2</sub>O was produced or transferred by winter wheat plants, these results indicated an important role for higher plant in N<sub>2</sub>O exchange. Identifying its potential contribution is critical for understanding agricultural ecosystem N<sub>2</sub>O sources.

### Introduction

Nitrous oxide is an atmospheric trace gas that contributes to global warming and the depletion of stratospheric ozone (IPCC, 1996), but its global budget remains poorly understood at present (IPCC, 2001). Perhaps the most important reason for the current uncertainty is the difficulty in measuring fluxes due to extraordinary spatial and temporal variability (Brumme et al., 1999; Khalil, 2000). Some studies have suggested that N<sub>2</sub>O input to the atmosphere from agricultural production as a whole has been previously underestimated (Kroeze et al., 1999; Mosier et al., 1998; Robertson et al., 2000). Understanding the role

of plants will help show the nature and extent of N<sub>2</sub>O emissions from agricultural ecosystem, and minimize the uncertainty in global N<sub>2</sub>O budget.

Much research has gone into assessing the role of growing plants in N<sub>2</sub>O production and emissions from agricultural systems (e.g., Chang et al., 1998; Grundmann et al., 1993; Haider et al., 1985; Müller, 2003). In general, the contribution of growing plants to ecosystem N<sub>2</sub>O emissions has been supported by three lines of evidence. First, plant roots facilitate N<sub>2</sub>O production in the soil. General denitrification models have elucidated that N<sub>2</sub>O production in soil is mainly controlled by the availability of nitrate, labile C compounds, and O<sub>2</sub> (Del Grosso et al., 2000), which is

\*E-mail: jwzou@rice.edu

greatly affected by the existence of growing plants (Conrad et al., 1983). Second, some studies have been devoted toward understanding a role of plant pathway in ecosystem  $N_2O$  emissions (e.g., Li and Chen, 1993; Yu et al., 1997). Mosier et al. (1990) reported that rice plants contributed to the efflux of  $N_2O$  from paddy soil. When the soil was flooded,  $N_2O$  emission was predominately through the rice plants (Yan et al., 2000). Chang et al. (1998) indicated that plant serves as a conduit to transport  $N_2O$  produced in soil to atmosphere based on the relationship between soil solution  $N_2O$  content and  $N_2O$  flux. Finally, recent evidence suggests that plants can emit  $N_2O$  under natural conditions, or plant  $N_2O$  emissions were directly detected in some studies. For example, it has been reported that a transgenic tobacco can emit  $N_2O$  when fed with  $^{15}N$ -labeled nitrate or nitrite (Goshima et al., 1999). Xu et al. (2001) found that significant amounts of  $N_2O$  were released from foliage of plants since there was an above-ground vertical concentration profile of  $N_2O$  within coniferous-deciduous mixed forests. The result obtained by Smart and Bloom (2001) demonstrated that wheat leaves emit  $N_2O$  during nitrate assimilation. A study with 17 plant taxa indicated that plant  $N_2O$  emission was common in plant tissues (Hakata et al., 2003).

So far, studies have centred around the plant  $N_2O$  pathway or plant  $N_2O$  production, while relatively few have focused on the whole seasonal contribution of growing plants to agroecosystem  $N_2O$  emissions. Measuring within a single stage of plant development, however, provides very little insight into the role of plants in the ecosystem  $N_2O$  emissions. Here we present the results from winter wheat outdoor pot and field experiments that employed the cutting plant method in combination with the static opaque chamber method. Comparing the ecosystem  $N_2O$  emissions before and immediately after cutting plants offers an approach to quantifying  $N_2O$  emissions from plants. We did not attempt to partition  $N_2O$  production in soil or plants, but instead concentrated on the overall contribution of plants to ecosystem  $N_2O$  emissions and the seasonal pattern of  $N_2O$  emissions from plants.

## Materials and methods

### *Pot and field experiments*

Both pot and field experiments were used to quantify  $N_2O$  emissions from plants at similar temperatures

and soil moisture, which are the main environmental factors important to  $N_2O$  emissions. We used pot experiment to minimize the differences in  $N_2O$  fluxes due to variability in soil moisture. The outdoor pot experiments were carried out at Nanjing Agricultural University, in Nanjing, Jiangsu province, China ( $31^{\circ}52'N$ ,  $118^{\circ}50'E$ ) during the 2001–02 and 2002–03 wheat-growing seasons. Soil for the experiment was taken from the top 20 cm of the profile in winter wheat croplands at Jiangsu Academy of Agricultural Sciences in Nanjing. This soil consisted of 4% sand, 45% silt, and 51% clay with an initial pH of 6.1. Total organic C and N were  $13.1\text{ g kg}^{-1}$  and  $1.1\text{ g kg}^{-1}$ , respectively. Pot experiments have been performed to investigate greenhouse gases emissions in rice-wheat rotation ecosystems previously and more details on methods can be found in Huang et al. (2002). Six pots were used in the 2001–02 season and 12 pots in the 2002–03 season. A local prevailing winter wheat cultivar (*Triticum aestivum* L.) was planted on November 5 in the 2001–02 season and on November 8 in the 2002–03 season. Nitrogen fertilizer was applied as urea at the rate of  $1.1\text{ g N pot}^{-1}$ , with a split of 55% as basal fertilizer, 25% on 115 days and 20% on 155 days after planting. There was no difference in fertilization regime between two seasons.

The field experiment was initialized on November 8 in the 2002–03 wheat-growing season. In order to create differences in biomass inside the gas sampling chamber, there were three experimental treatments designed to represent low, normal and high planting densities. In the field, 27 pots with the bottom removed were installed as gas flux collars and evenly distributed in the three treatments. We checked the number of seedlings and tillers in the pot according to density treatments on 30 days and 110 days after planting, respectively. Urea was used at the rate of  $200\text{ kg N ha}^{-1}$ , split as 30% as basal fertilizer, 45% on 110 days and 25% on 155 days after planting. Phosphorus and potassium were applied as the basal fertilizer at the local rate and no additional organic manure was incorporated in the field. After going through winter stages, wheat plants entered the main active growth phases at the end of February (about 120 days after planting).

### *Gas samples and measurements*

$N_2O$  emissions from plants are typically determined by the cutting plant method (Chen et al., 1999; Müller, 2003; Yan et al., 2000). We performed a preliminary study that showed no obvious changes in soil  $N_2O$

fluxes at 0, 10, and 24 h after cutting plant, which suggested that changes to soil N<sub>2</sub>O emissions by cutting plants were negligible. This method assumes that N<sub>2</sub>O emissions from plants can be quantified as the difference in soil-crop ecosystem N<sub>2</sub>O fluxes before and immediately after cutting plant. For CO<sub>2</sub>, this difference represents plant shoot dark respiration since plant photosynthesis in an opaque chamber is interrupted while gas sampling (Zou et al., 2004). For the group-cutting performed in the 2001–02 pot experiment, in which biomass in each pot was cut at ground level three times, it was assumed that once was about 1/3 total biomass. The fluxes of N<sub>2</sub>O emissions from soil-wheat system were measured each time before and after 1/3 of biomass were removed from a pot. We found that ecosystem N<sub>2</sub>O fluxes gradually decreased with plants removed by group-cutting. To further investigate N<sub>2</sub>O emissions from plants, one-off cutting was conducted to remove the whole biomass in each pot in the 2002–03 pot and field experiments. During the main active growth phases, measures were taken twice for the 2001–02 pot experiment, four times and three times for the 2002–03 pot and field experiments, respectively (Table 1). Sampling date was selected on the basis of soil temperature and soil moisture. Each time three pots were used as replicates for gas samples. While taking gas samples, the opaque chamber was placed over the vegetation with rim of the chamber fitted into the groove of pot. The sampling chamber was 100 cm high and wrapped in a layer of sponge and aluminium foil to minimize temperature change during the period of sampling.

Nitrous oxide and CO<sub>2</sub> mixing ratios were detected by a modified gas chromatography (Agilent 4890D) with an electron capture detector and a hydrogen flame ionization detector (Wang and Wang, 2003; Zou et al., 2002). Nitrous oxide was separated by 2 stainless steel columns (column-1 with 1 m length and 2.2 mm i.d., column-2 with 3 m length and 2.2 mm i.d.) that packed with 80–100 mesh porapak Q, and detected by ECD. Carbon dioxide was separated by one stainless steel column (2 m length and 2.2 mm i.d.) that packed with 50–80-mesh porapak Q, afterwards hydrogen reduced CO<sub>2</sub> to CH<sub>4</sub> in a Nickel catalytic converter at 375°, and detected by FID. Oven was operated at 55 °C, ECD at 330 °C and FID at 200 °C, respectively. Detection limit for N<sub>2</sub>O flux is 0.41 mg N<sub>2</sub>O-N m<sup>-2</sup> day<sup>-1</sup> at 1000 hPa and 25 °C (Wang and Wang, 2003). Flux was determined from the slope of the mixing ratio changes in the three samples, taken at 0, 10 and 20 min

after chamber closure. Average N<sub>2</sub>O flux and standard deviation were calculated from three replicates.

Air temperature inside the chamber was recorded with each set of emission measurements and soil moisture was measured by soil water measurement instrument (MPM-160). In this study, air temperature was 15 ± 3 °C and soil moisture was about 75% WFPS (Water filled pore space) on all gas sampling dates. This should have minimized differences in N<sub>2</sub>O fluxes due to variability in air temperature or soil moisture.

The biomass of winter wheat was determined by oven dried to constant weight at approximately 70 °C. The C and N contents of plant and soil was determined by the protocol described in the Chinese Soil Society Guidelines (Lu, 2000).

## Results

### *Seasonal contribution of N<sub>2</sub>O emission from plant*

Soil-wheat ecosystem N<sub>2</sub>O emissions before and after group-cutting in the 2001–02 pot experiment are shown in Figure 1. Total N<sub>2</sub>O fluxes gradually decreased from 3.59 to 1.19 mg N<sub>2</sub>O-N m<sup>-2</sup> day<sup>-1</sup> on 150 days and from 3.60 to 0.69 mg N<sub>2</sub>O-N m<sup>-2</sup> day<sup>-1</sup> on 176 days when the wheat plants were removed from ecosystem (Figure 1). Plotting N<sub>2</sub>O emissions from plants against biomass produced a significant linear relationship (Figure 2). This relationship held for the 2002–03 pot and field experiments through one-off cutting as well (data not shown). On the other hand, analysis of variance (ANOVA, SYSTAT 10) showed significant differences in the soil-wheat ecosystem N<sub>2</sub>O amounts among the three field planting density treatments ( $p = 0.011$ ). After shoots were wholly removed, however, soil N<sub>2</sub>O fluxes did not significantly vary with density ( $p = 0.409$ ). This suggests that plants greatly contributed to ecosystem N<sub>2</sub>O emissions.

The contribution of plants to the soil-wheat ecosystem N<sub>2</sub>O fluxes was different among the three treatments on the same measuring date (Figure 3). Within the same planting density treatment, on the other hand, its contribution increased with winter wheat development (Figure 3). Seasonal contribution to the ecosystem N<sub>2</sub>O emissions was, on average, 25%, ranging from 10% for low density treatment at tillering through 62% for high density treatment at heading stage (Figure 3). The contribution reached 75% at the flowering stage in the pot experiments. Overall, we

Table 1. Gas sampling date in the 2001–02 pot and the 2002–03 pot and field experiments

Experiment	Whole season (days)	Sampling date (days)	Normalized stage
2001–02 pot	195	150, 176	0.77, 0.90
2002–03 pot	192	133, 152, 162, 172	0.70, 0.80, 0.85, 0.90
2002–03 field	203	132, 153, 166	0.65, 0.75, 0.82

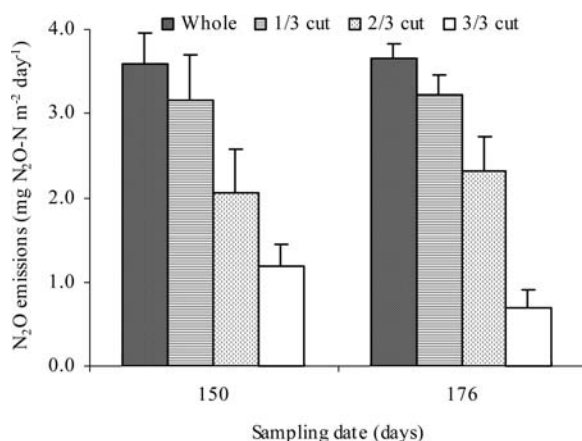


Figure 1. The effect of group-cutting plant on  $N_2O$  emissions (mean  $\pm$  1 SD) from soil-winter wheat ecosystem in the 2001–02 pot experiment. The '1/3 cut' means ecosystem  $N_2O$  emissions after 1/3 wheat biomass removed.

found that the contribution of  $N_2O$  emissions from plants was comparable to that of plant autotrophic respiration to ecosystem  $CO_2$  emissions. In short, the contribution of plants to agricultural ecosystem  $N_2O$  emissions increased with biomass which varies with planting density or plant development.

#### Quantifying $N_2O$ emission from plant

Plant respiration rates are often expressed in terms of a respiratory coefficient ( $R_E$ ,  $g\ CO_2-C\ g^{-1}\ C\ day^{-1}$ ), i.e.,  $CO_2-C$  emission in grams through respiration from per gram carbon of plant dry matter per day. The difference in ecosystem  $CO_2$  effluxes before and after plant are removed represents shoot dark respiration since plant photosynthesis in an opaque chamber is interrupted while carbon fluxes are being measured. Thus,  $R_E$  is one of the indexes of plant dark respiratory intensity. Similarly, we defined a  $N_2O$  emission coefficient ( $N_2O_E$ ,  $mg\ N_2O-N\ g^{-1}\ C\ day^{-1}$ ) as  $N_2O-N$  emission in milligram from per gram carbon of shoot dry matter within a day to quantify  $N_2O$  emissions from plants.  $N_2O_E$  was calculated from the relationship between  $N_2O$  emissions from plants and biomass

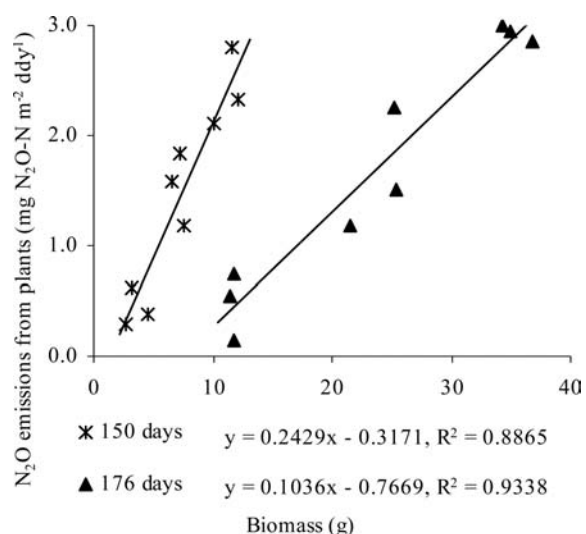


Figure 2. The dependence of  $N_2O$  emissions from plants on plants biomass on 150 and 176 days in the 2001–02 pot experiment.

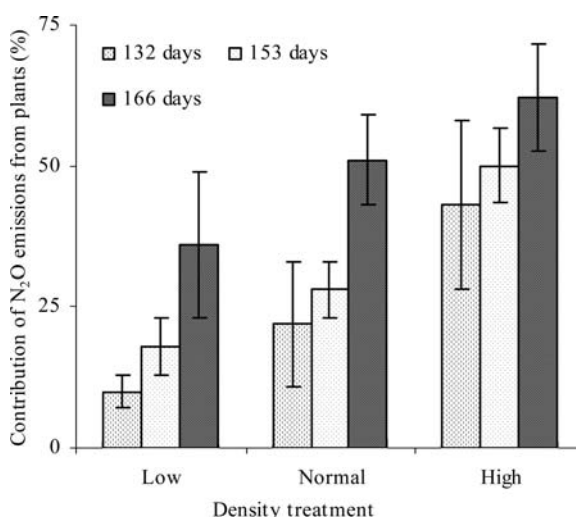


Figure 3. The dependence of the contribution of  $N_2O$  emissions (mean  $\pm$  1 SD) from plant to the total ecosystem fluxes on field planting density at three stages in the 2002–03 season.

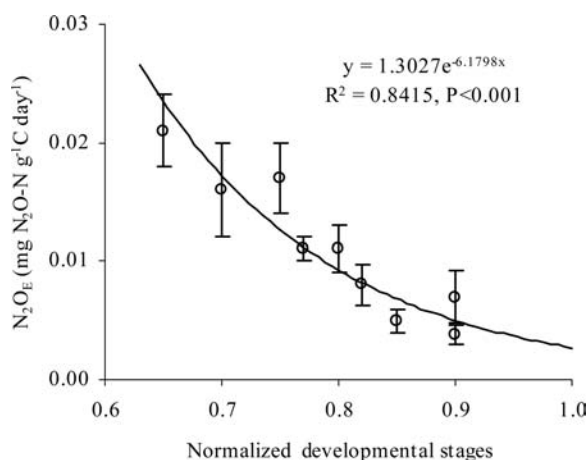


Figure 4. The temporal trend of  $\text{N}_2\text{O}$  emissions from plants ( $\text{N}_2\text{O}_E$ , mean  $\pm 1$  SD) during the main growing stages of winter wheat.  $\text{N}_2\text{O}_E$  is defined as  $\text{N}_2\text{O}$ -N emission in milligram from per gram carbon of shoot dry matter within a day. Developmental stage of winter wheat was described as the ratio of days after planting to days in the whole season.

(e.g., Figure 2). Since there was a difference in plant growth rate among the 2001–02 pot, 2002–03 pot and field experiments (Table 1), winter wheat developmental stage was normalized as the ratio of days after planting to days in the whole season to describe the temporal dynamics of  $\text{N}_2\text{O}_E$ . There was no significant difference of  $\text{N}_2\text{O}_E$  among different pots or the three field treatments on the same measuring date, but  $\text{N}_2\text{O}_E$  varied consistently over the wheat growing season (Figure 4).  $\text{N}_2\text{O}_E$  decreased with wheat development, although the contribution of plant to the total flux increased with wheat biomass expansion (Figure 3). Average  $\text{N}_2\text{O}_E$  varied considerably with a 5-fold difference between  $0.021 \text{ mg N}_2\text{O-N g}^{-1}\text{C day}^{-1}$  at winter wheat tillering and  $0.004 \text{ mg N}_2\text{O-N g}^{-1}\text{C day}^{-1}$  at flowering stage (Figure 4). The seasonal variation of  $\text{N}_2\text{O}_E$  was in agreement with that of plant dark respiratory coefficient ( $R_E$ ). There was a pronounced linear relationship between  $R_E$  and  $\text{N}_2\text{O}_E$  within pot and field data sets (Figure 5).

## Discussion

It is generally accepted that there are two mechanisms for the  $\text{N}_2\text{O}$  efflux from plants:  $\text{N}_2\text{O}$  derived from soil is transported by plant, or  $\text{N}_2\text{O}$  is directly produced by plant itself during plant N assimilation. Nevertheless, it is difficult to distinguish which one is the dominant mechanism or whether these two mechanisms are

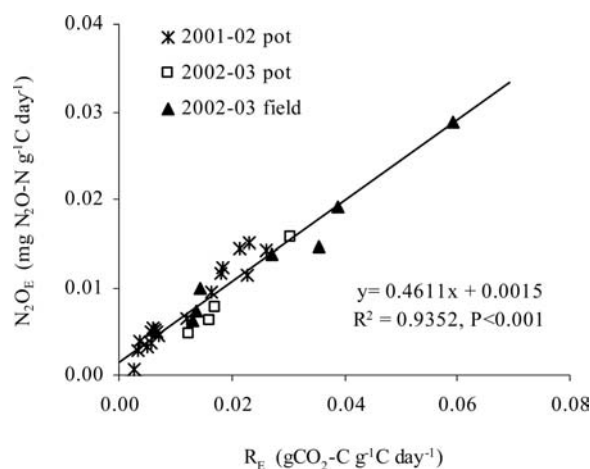


Figure 5. Correlation between  $\text{N}_2\text{O}_E$  and  $R_E$  in soil-wheat ecosystem.  $R_E$  is represented as  $\text{CO}_2$ -C emission in grams through respiration from per gram carbon of plant dry matter per day.  $\text{N}_2\text{O}_E$  is defined as  $\text{N}_2\text{O}$ -N emission in milligram from per gram carbon of shoot dry matter within a day.

in operation at the same moment in field conditions (Müller, 2003; Xu et al., 2001). A pot experiment by Chen et al. (1999) showed that  $\text{N}_2\text{O}$  emissions from rye grass (*Lolium perenne* L.) was related to plant nitrate content although it was not clear whether  $\text{N}_2\text{O}$  was produced by plants themselves or the rye grass only served as a conduit for  $\text{N}_2\text{O}$  produced in the soil. Nitrification and denitrification in the soil are the major  $\text{N}_2\text{O}$  sources, while, several microbial organisms in the plant that do not nitrify or denitrify can also produce  $\text{N}_2\text{O}$  in the process of inorganic N assimilation (Hutchinson and Livingston, 1993). Any enzymatic nitrogen transformation through the +2 to +1 oxidation state may generate  $\text{N}_2\text{O}$  (Firestone and Davidson, 1989). Nitrite assimilation in chloroplasts can generate intermediates capable of reacting to produce  $\text{N}_2\text{O}$  (Dean and Harper, 1986).

In fact, the process of inorganic N assimilation in which  $\text{N}_2\text{O}$  produces interacts with both photosynthesis and respiration. The ATP and reductant required to assimilate N may be provided by photosynthesis, respiration or both (Turpin et al., 1997). For example, Smart and Bloom (2001) reported that wheat leaf  $\text{N}_2\text{O}$  emissions were correlated with leaf nitrate assimilation activity, as measured by the assimilation quotient (the ratio of  $\text{CO}_2$  assimilated to  $\text{O}_2$  evolved), when nitrate was abundant. As well, N assimilation requires carbon skeletons that are provided by respiration. While our understanding of the regulatory mechanisms controlling carbon partitioning in response to N assimilation is still incomplete, it is obvious that N assimila-

tion depends upon increased respiratory carbon flow (Turpin et al., 1997). Moreover, a capacity to use the tricarboxylic acid (TCA) cycle and the oxidative pentose phosphate (OPP) pathway reductant rather than photosynthetic electrons for the assimilation of  $\text{NO}_3^-$  is clearly demonstrated by  $\text{NO}_3^-$  assimilation in darkened photosynthetic tissues of higher plants (Reviewed by Huppe and Turpin, 1994). This suggests that  $\text{N}_2\text{O}$  production in plant N assimilation also occurs in the absence of plant photosynthesis.

The results of this study did not prove that  $\text{N}_2\text{O}$  was actually produced in the plants, but demonstrated that this is a reasonable possibility, and could have been responsible for the observed  $\text{N}_2\text{O}$  emissions from wheat plants. The correlation between  $\text{N}_2\text{O}$  emissions from plants ( $\text{N}_2\text{O}_E$ ) and plant respiratory coefficient ( $R_E$ ) in this study indicated that  $\text{N}_2\text{O}$  emissions from plants might be associated with plant respiration. Hakata et al. (2003) also found that there was more than a 58-fold variation in the ' $\text{N}_2\text{O}$  Emission' in the 17 plant taxa, which suggested plants  $\text{N}_2\text{O}$  emissions might be involved with plant intrinsic physiological characteristics. On the other hand, night-time plant transpiration appears to be potentially widespread in plants. There is convincing evidence that stomata are not fully closed and stomatal transpiration is still happening in the dark (Synder et al., 2003). So, it is likely that  $\text{N}_2\text{O}$  emissions from plants were also partly produced in the soil due to plant conduit function.

In order to construct a global biogenic  $\text{N}_2\text{O}$  budget, scientists are interested in assessing the total contribution of plants in agricultural ecosystem  $\text{N}_2\text{O}$  emissions as well as determining where  $\text{N}_2\text{O}$  is produced. This study could not show whether  $\text{N}_2\text{O}$  was produced or transferred by winter wheat plant, but it was clear that soil-crop system  $\text{N}_2\text{O}$  emissions were greatly affected by plants. The seasonal amount of  $\text{N}_2\text{O}$  emissions from plants was equivalent to 0.23% of plant N released as  $\text{N}_2\text{O}$ . The fluxes of  $\text{N}_2\text{O}$  emissions from plants varied between 0.3 and 3.9  $\text{mg N}_2\text{O-N m}^{-2} \text{ day}^{-1}$  and its seasonal amount was responsible for 25% of total ecosystem  $\text{N}_2\text{O}$  emissions in this study. A chamber study showed that  $\text{N}_2\text{O}$  emissions from rye grass varied between 0 and 2.8  $\text{mg N}_2\text{O-N m}^{-2} \text{ day}^{-1}$  while ecosystem  $\text{N}_2\text{O}$  emissions varied between 0.8 and 13.3  $\text{mg N}_2\text{O-N m}^{-2} \text{ day}^{-1}$  (Chen et al., 1999). In another study, *Linum perenne* canopies contributed as much as 50% of  $\text{N}_2\text{O}$  flux to the atmosphere (Anderson and Hopkins, 1997). Li et al. (2002) found that aerodynamic flux measurements over some crop fields were about 12% higher

than those measured by static opaque chamber, which was mainly ascribed to the contribution of plants. An earlier study by us on the influence of planting density on  $\text{N}_2\text{O}$  fluxes indicated that plants contributed to  $\text{N}_2\text{O}$  emissions in winter wheat fields (Huang et al., 2001). The results of a rice paddy study also showed that  $\text{N}_2\text{O}$  emissions from plots with plants included was increased by 37% in comparison with plots without plants (Zou et al., 2003).

Our results have demonstrated that the role of higher plants in  $\text{N}_2\text{O}$  exchange can be substantial and its potential contribution makes it vital to identify agricultural ecosystem  $\text{N}_2\text{O}$  sources. To accurately quantify  $\text{N}_2\text{O}$  emissions from agroecosystems, therefore,  $\text{N}_2\text{O}$  flux measurements based on static chamber techniques should be sure that appropriate vegetation with normal growth status is covered in the chamber. Further, it is crucial that  $\text{N}_2\text{O}$  emissions from plants are investigated in various crop systems.

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